PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A01H 1/00, A01G 9/02
C05F 11/08, C12N 5/00
C12P 19/04, C12N 1/00

A1

(11) International Publication Number: WO 90/15527

(43) International Publication Date: 27 December 1990 (27.12.90)

(21) International Application Number: PCT/US90/02859

(22) International Filing Date: 21 May 1990 (21.05.90)

(30) Priority data:
365,585 13 June 1989 (13.06.89) US

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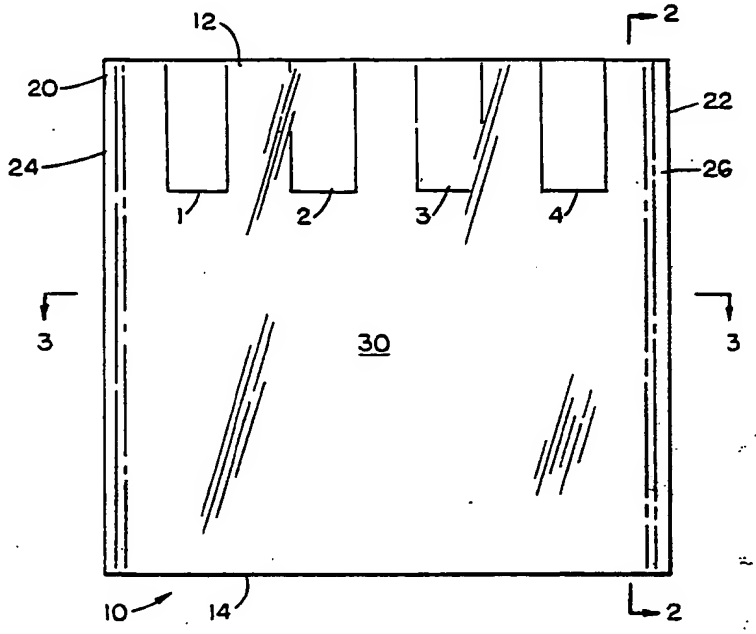
(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CM (OAPI patent), DE*, DE (European patent)*, DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL, NL (European patent), NO, RO, SD, SE, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent).

Published
With international search report.

(54) Title: INTEGUMENT WITH BREAKABLE INNER CONTAINERS

(57) Abstract

Disclosed is an integument (10) having a gas-permeable outer membrane (12) which is contaminant and liquid impermeable, and which includes one or more internal containers (1). The internal containers (1) can be opened from the outside of the integument (10), to release their contents, without opening the integument outer membrane (12) or breaching the integrity thereof. The system is designed so that the outer membrane (12) houses a biological product and the inner containers (1) house a bacteria media, or other product which has an effect on the biological product when the inner container (1) is opened. In an alternate system, the biological product is housed in the inner container (1) and the outer membrane (12) holds a product which affects that biological product. The system can be advantageously employed in micropropagation, seed growth or inoculation of plant material, bacterial culturing, or crystal growth, as well as in many other applications.



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INTEGUMENT WITH BREAKABLE INNER CONTAINERS

RELATED APPLICATIONS

This application is a continuation-in-part of U. S. Application Serial No. 207,405 filed on June 14, 1988, and entitled "Integument and Method for Micropropagation and Culturing Organic Material", which is a continuation-in-part of U. S. Application Serial No. 021,408 filed March 4, 1987, and entitled "Integument and Method for Micropropagation and Tissue Culturing."

FIELD OF THE INVENTION

The invention relates to an integument having a gas-permeable membrane which is contaminant and liquid impermeable, and which includes one or more internal containers. The internal containers can be opened from the outside of the integument, to release their contents, without opening the integument.

BACKGROUND OF THE INVENTION

In most fields of biotechnology, it is necessary to maintain a sterile environment around a growing culture. This is true whether the culture is living organic material such as bacteria, human or animal tissue, cells or cell lines, or plant tissue. In certain cases, a sterile environment may also be desired for plants grown from seeds, in order that contamination is minimized or prevented.

Typically, sterility is achieved by cleaning and sterilizing the initial culture sample and then culturing the sample in a sterilized vessel. Such vessels can include test tubes, plates with wells, or petri dishes.

Prior art sealed containers, as for example test tubes, are deficient in that they restrict gas exchange (of oxygen and carbon dioxide) required by most cultures for life and growth. Accordingly, although the test tubes are covered to prevent entry of contaminants which can ruin the cultures, the covers provide limited gas exchange. Typically, the test tube covers include a rubber stopper with a hole packed with cotton. Alternatively, a loose fitting cap with baffled slits is used, wherein limited gas exchange is permitted around the loose fit and through the baffles.

Cultures grown in a well plate or petri dish are housed in incubators where the gas mixtures and pressures are monitored and maintained to minimize contamination. Thus, the laboratory often includes either a culture room with an air filter system or an incubator to combat air-borne contaminants. Further, the laboratory is kept extremely clean and workers may be provided with protective gear, such as face

masks, to reduce the introduction of contaminants by workers.

The foregoing systems are expensive and despite the best efforts at maintaining sterile conditions, some contamination nevertheless occurs. In addition, glass containers are expensive, subject to breakage, and occupy a good deal of space in the laboratory. If the glass containers are sterilized after use to reduce the cost of replacement, this increases the chances of contamination because sterilization may be incomplete.

Contamination can be introduced into the prior art container at any time the container is opened to add anything to the culture. Such additives could include growth media or perhaps other living matter which affects growth or development. Special care must be taken to prevent the introduction of contaminants. In addition it would be desirable to pre-measure the amount of additive to be introduced into the growing culture, particularly if the process is being carried out commercially. This would help prevent errors in the quantity introduced, which could easily occur if an unskilled worker was performing the addition in a commercial process.

The advantages of the invention in solving the foregoing deficiencies of the prior art will appear from the following description.

SUMMARY OF THE INVENTION

The invention includes an integument having one or more internal containers. The outer integument is made of a gas-permeable, contaminant and liquid impermeable membrane. The internal containers can be made of a material which is permeable, semi-permeable or impermeable to gases and liquids. The outer integument and internal containers are preferably made of a high density polyethylene such as Chevron 9650.

A biological product is housed in the integument outside the internal containers. The internal containers include a predetermined amount of a selected material which effects the biological product upon mixing. The selected material may be a biological agent, growth factor, activator, fixative, hormone, nutrient or other material which affects the biological product. The internal containers can be broken to release their contents into the integument, and to mix the contents with the biological product. The internal containers can be opened at a predetermined time to release their contents and mix with the biological product without opening the

integument. The internal container is opened by applying pressure on the integument's outer surface in the area of the internal container until the container breaks. Thus, the mixing can occur in pre-measured amounts and in a contaminant-free environment.

The preferred membrane allows gas transmission and light transmission, but is impermeable to contaminants and liquids. Light transmission is particularly desirable for the growing of seeds, plant tissue, plants, or other vegetative matter. Because the membrane of the integument has a relatively low rate of moisture vapor transmission, the media tends not to desiccate. When the inner container(s) is broken and its contents are introduced into the media, the relatively high gas exchange rate through the membrane enhances the growth rate of the biological product, whether that product is vegetative matter, bacteria, fungus, tissue, cells, or any other type of product.

There can be one or more innermost containers housed within the inner container(s). This system would function substantially the same as those described above, with one or more of the containers housing a biological product, and the other

containers housing a material which affects that biological product.

Other objects and advantages of the present invention will appear from the following drawings and description.

BRIEF DESCRIPTION OF THE DRAWINGS

For a detailed description of the preferred embodiment of the invention, reference will now be made to the accompanying drawings, wherein:

Figure 1 depicts a frontal view of an integument of the present invention with several breakable inner containers;

Figure 2 depicts a partial elevation cross-sectional view of the integument of Figure 1 taken along plane 2-2 as shown in Figure 1 with the material of the integument enlarged;

Figure 3 depicts a partial top view of the integument of Figure 1 with the material of the container enlarged;

Figure 4 depicts a view of the integument of Figures 1-3;

Figure 5 is a plan view of an integument of the invention having several chambers bonded to each other, with each chamber housing a breakable inner container;

Figure 6 is a plan view of an integument of the invention suitable for growing a plant therein from seed;

Figure 7 is a side view of the integument of Figure 6;

Figure 8 is a schematic drawing of a process for the manufacture of the polymer used in the membrane of the invention;

Figure 9 is a schematic view of the molecular structure of various polyethylenes;

Figure 10 is a graph showing the moisture vapor transmission rate of three different polyethylenes versus film thickness;

Figure 11 is a graph of the oxygen transmission rate of the three polyethylenes in Figure 10 versus film thickness; and

Figure 12 is a graph of the carbon dioxide transmission rate of the three polyethylenes in Figures 10 and 11 versus film thickness.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Referring initially to Figures 1-4, there is shown an integument 10 of the present invention. Integument 10 is made of membrane 12 which, when sealed, completely and entirely surrounds and encloses the contents, and one or more inner pouches

or containers such as containers 1, 2, 3, and 4, respectively, from the ambient environment.

Integument 10 is made by folding membrane 12 over at 14 such that two sides 16, 18 are formed. Sides 16, 18 are heat sealed at 24, 26 along the entire length thereof, perpendicular to fold 14 and adjacent to longitudinal edges 20, 22 of membrane 12 so as to form a cellule 30. The cellule 30 forms an expandable chamber 31. The cellule 30 has an average volume which can vary widely, depending on the specific use to which it is put. Thus, cellule 30 may be of various sizes. The cellule 30 has at least initially, an open end 28 formed by the terminal edges 32, 34 of membrane 12. End 28 serves as a port of entry for chamber 31 of cellule 30 for receiving the contents. Upon the insertion of the contents, terminal edges 32, 34 are heat sealed at 36 to completely enclose and seal the chamber 31.

As can also be appreciated, rather than being made of a single folded membrane 12, integument 10 can be made of two individual and separate pieces of material such as a base sheet of material and a frontal sheet of material. In this alternative embodiment, the bottom of cellule 30 is formed by heat sealing the frontal material to the base material near the lower terminal edges thereof as

distinguished from the fold at 14 where a single piece of material is used as described with respect to Figures 1-4. Composite materials may be formed to take advantage of the strength of one material and the permeability to oxygen and carbon dioxide of another, as for example. The integument 10 can also be made tubular and cut into predetermined lengths with one end closed by heat sealing.

The integument 10 may include an upper flap or band formed at its upper end in heat sealed portion 36 for the purpose of suspending the integument 10 in the vertical position. Suitable connection means such as apertures 38, 40 in heat sealed portion 36 may be provided with attachment means such as drapery hooks or S-hooks to suspend integument 10 vertically if desired.

The material for membrane 12 of integument 10 is critical to providing the necessary environment for housing living organic material within chamber 31. In particular, it is important to achieve optimum gas exchange and light transmission to permit the necessary biochemical activity conducive to life. The membrane 12 must readily pass oxygen from the atmosphere into the chamber 31 of the integument 10 to be diffused through the contents thereof for use by the organic material in metabolic processes and

also pass carbon dioxide out of the chamber 31 of integument 10. Thus, the membrane 12 of integument 10 is made of a semi-permeable and translucent material which permits gas transfer therethrough, described in detail below.

Upon sealing the chamber 31 of cellule 30, the contents are completely enveloped and enclosed from the ambient atmosphere and environment so as to prevent any introduction of contaminates. Gas exchange is permitted between the contents and the atmosphere of the ambient environment to permit the absorption of oxygen and the release of carbon dioxide. The material of membrane 12 is also translucent for most living organic material to receive the necessary light for life and growth.

In operation, the contents are inserted through opening 28 and into chamber 31 of integument 10. Thereafter, the opening 28 may be heat sealed at 36 for closing the chamber 31 of integument 10. If desired, a means may be provided, such as a tube, for introducing additional material to the integument 10 after the same has been filled.

Housed within integument 12 are a series of inner containers 1 to 4, respectively. Each inner container is smaller than integument 14, but is also substantially rectangular in shape. Inner containers

1 to 4 are sized for the particular contents housed therein. Each inner container is made of two pieces of material, heat sealed on four sides. The uppermost edge of each inner container, respectively, is bonded to the upper heat sealed side 36 of integument 10. In this embodiment, the bonding is accomplished by heat sealing the upper edge of each inner container between the two sides 16, 18 of membrane 12. However, other methods of attachment are also possible and within the scope of the invention. Alternatively, the inner containers need not be attached at all but could float freely within the outer integument 10. Further, membrane 12 could house a greater or lesser number of inner containers, and as few as only one inner container. Another alternative is to have further innermost containers housed within the inner containers, as explained below.

Inner containers 1-4 are breakable from outside the integument 10. The inner containers are made of a material which will burst under pressure. A flat edge may be pressed down on one end of an inner container and then slid along a flat surface capturing air or other gas within the inner container until a bubble is formed within the inner container. By continuing to press the flat edge against the

trapped bubble, the material of the inner container will break or burst releasing the contents of the inner container into the outer integument 10.

Referring now to Figure 5, another embodiment of an integument 40 of the invention is shown. Integument 40 has a series of chambers 42, 44, 46, 48 and 50, respectively, formed side by side. Integument 40 has an outer membrane 52, similar to outer membrane 12 of integument 10. Outer membrane 52 is folded over on side 54, and heat sealed on the two perpendicular sides 56, 58, and upper side 60. Each chamber is formed by heat sealing along a line substantially parallel to sides 56 and 58. Each chamber is substantially rectangular in shape.

Inner containers, 62, 64, 66, 68 and 70, are situated within each chamber, 42, 44, 46, 48 and 50, respectively. Each inner container is bonded to the upper side 60 of integument 40. In this embodiment, the bonding is accomplished by heat sealing the upper edge of each inner container between the two pieces of membrane 52 which form the upper side 60. However, other methods of attachment are also possible and within the scope of the invention. Alternatively, the inner containers need not be attached at all but could float freely within the outer membrane 52. Again, it is possible to have

innermost containers within the inner containers.

Referring now to Figures 6 and 7, an integument 100 of the invention is shown. Integument 100 has an outer membrane 101 which is folded into the shape of a cubical bag with folded quarter panels on one section thereof. Integument 100, and the manner of making it, is described with particularity in co-pending U. S. Application Serial No. 021,428. It should be noted that integument 100 has two chambers. There is an upper chamber 102 in which plant stem and leaf can grow, and a lower chamber 104 which can house a media and plant roots. A narrowed neck portion 106 separates the two chambers. Integument 100 is heat sealed along the lowermost edge 108 thereof.

Attached to the upper edge of upper chamber 102 is an inner container 110. In this embodiment, the attachment is accomplished by gluing the upper edge of each inner container between to the inside surface of upper chamber 102. However, other methods of attachment are also possible and within the scope of the invention. Alternatively, the inner containers need not be attached at all but could float freely within the upper chamber 102. Again, it is possible to house innermost containers within the inner containers.

The outer membranes 12, 52 and 101 are all preferably formed of a material that is gas permeable, liquid impermeable, impermeable to contaminants such as virus particles, light transmissible, and resistant to high temperatures. However, some of these properties can vary depending on the particular application desired for the membrane. For example, where the integument is used for germinating seeds before planting the seeds in a non-sterile environment, contaminant impermeability is not a concern. This is described further below.

The material of the membranes is translucent and allows the passage and diffusion therethrough of light rays, having at least the wavelengths of 400 to 750 nanometers, so as to be useful in plant production. Individual wavelengths of light in the range of 400 to 750 nanometers are required by individual photosynthetic agents, such as the chlorophylls, to provide the reactions necessary for life and growth. The reduced thickness of the material for the outer membranes and the uniformity of molecular structure formed in part by the extrusion process for the material for membranes permits greater light transmission. The approximate 1.25 mil thickness of the material for the membranes substantially enhances the amount of light and the

various individual wavelengths of light which are received by the culture. It is important that each wavelength of light necessary for each photosynthetic agent to react pass through the integument. The uniformity and light cross-linking of the molecular structure of the material for the membranes provides a pathway of lesser resistance for light.

Certain microorganisms live and grow anaerobically. The cellule for culturing these microorganisms can be made from less permeable materials so as to preclude a gaseous interchange between the ambient environment and the organic material. Similarly, some microorganisms prefer or require the absence of light. When culturing such microorganisms, an opaque material can be used for the membrane.

The material for the membranes may be in the range of 0.1 to 5.0 mils thick but is preferably between 1 and 2 mils thick, and it is most preferred that the material of the membranes have a thickness of 1.25 mils. If the membrane material is much thinner than 1.0 mil, handling the membrane is made more difficult, as the opposing surfaces of the material of the membrane tends to adhere to each other when formed in such thin materials. The reduced thickness of the material for membrane 12 and

the uniformity of molecular structure formed in part by the extrusion process for the material for the membranes enhances gas transmission. The approximate 1.25 mil thickness of the material for the membranes substantially enhances the amount of light and the various individual wavelengths of light which are received by the culture. If the membrane material is much thinner than 1.0 mil, handling the membranes is made more difficult, as the opposing surfaces of the material of the membranes tend to adhere to each other when formed in such thin films.

Polyethylene has been found to be the preferred material for the membranes. Polyethylene, a thermal plastic resin, is melt formable and can be produced as a thermal plastic film. Polyethylene film is pliable and collapsible such that it can be stored and shipped in rolls. Further, polyethylene is inexpensive, as compared to glassware, such that it is disposable.

Linear polyethylene is preferred over branched polyethylene. Linear polyethylene is distinguished from branched polyethylene by its method of manufacture. Linear polyethylene is polymerized in reactors maintained at pressures far lower than those required for branched polyethylene. Linear polyethylene encompasses ultra-low-density, linear

low-density, high-density, high-molecular-weight high-density, and ultrahigh-molecular-weight polyethylene, which structures are shown schematically in Figure 9. Linear polyethylene density varies with the quantity of comonomer used with ethylene. The comonomer forms short-chain branches along the ethylene backbone. The greater the quantity of comonomer the lower the density of the polymer.

Polyethylene resins are characterized by their density. Linear low-density polyethylene has a density generally between 0.916 and 0.940 grams per cubic centimeter. High-density polyethylene is characterized by densities in the range of 0.940 to 0.965, with 0.962 grams per cubic centimeter as the practical upper limit. Polyethylene resins are also characterized by a particular resin's molecular weight which is an inverse function of its melt index. Thus, melt index is a basic physical characteristic of a particular polyethylene resin. Most commercial high-density polyethylene resins have molecular weights in the range of 50,000 to 250,000. High-density polyethylenes are available with molecular weight distributions ranging from narrow to broad. Molecular weight distribution is generally expressed by the measurement of the resin's ability

to flow under controlled conditions, i.e. its melt index. High-density polyethylene resins have melt indexes ranging from 0.04 to 75 grams per 10 minutes.

High-density polyethylene is preferred over low-density polyethylene in most applications. High-density polyethylene is a highly crystalline, lightweight thermoplastic. High-density polyethylene is translucent and semipermeable. Its principle characteristics are stiffness, good environmental stress-crack resistance, chemical resistance, toughness (even at low temperatures), dielectric properties, water vapor impermeability, low-temperature properties, and relatively high softening temperature. Its structure is closest to that appearing beside "HDPE" in Figure 9.

There are three basic manufacturing techniques for the production of high-density polyethylene: particle form slurry reactors, tandem reactors, and gas phase reactors. The particle form slurry reactor method is the most widely used and includes a supported transition metal catalyst slurried in a diluent that is circulated in a closed-loop reactor at a low pressure and low temperature. Ethylene, which is dissolved in the diluent, reacts with the catalyst to form solid high-density polyethylene

particles. The particles are dried, combined with additives, and extruded as pellets.

High-density polyethylene can be processed by all melt forming methods, including extrusion, injection molding, rotational molding, blow molding, and powder coating. The primary property affecting processing is melt index. The largest amount of high-density polyethylene is consumed in blow molding applications. High-density polyethylene resins with a melt index in the 0.1 to 1.0 range generally are used. See Modern Plastics Encyclopedia, published by McGraw-Hill, Inc. (1988) incorporated herein by reference.

Other materials which have the desired properties for particular applications, e.g., light translucency, gas permeability and contaminant impermeability, are also available for the outer membranes. For example, certain translucent low density polyethylene is suitable and even allows greater gas permeability than the preferred high density polyethylene; however, such low density polyethylene cannot withstand the high temperatures of the autoclave (250° at 15 p.s.i. for example) and the material melts or is otherwise deformed in the process. Accordingly, if such materials are used for the

membranes they must be sterilized through other means.

It should also be noted that although the focus of the foregoing discussion has been on the outer membranes, the membranes which form the inner containers need not have the same properties as the outer membranes, and therefore need not be made of the same material, although the same material is suitable. In most applications, the inner containers do not house a growing plant or organism, and therefore gas permeability is not a concern. In general, the inner containers only need to have the property of liquid impermeability, in that they will house liquids. However, in most applications a sterile environment is needed within the integument. Therefore it is preferable if the inner container is formed of a material which can withstand autoclave sterilization temperatures. Most high density polyethylenes are suitable.

Turning again to the outer membranes, the high density polyethylene at a thickness of 1.25 mils forms a molecular structure during the extrusion process which is especially useful. The high density polyethylene is made from linear crystalline polymers of suitable molecular weight with high tensile strength and extension modulus, a high degree of

symmetry, strong intermolecular forces and a controlled degree of cross-linking between layers. The cross-links between adjacent layers of polymers are introduced to prevent the polymeric chains from slipping under applied stress. The lightly cross-linked adjacent uniform layers of polymers of the high density polyethylene for the outer membranes form interstices therebetween which allow the preferred diffusion and osmosis therethrough for the desirable gas exchange and light transmission between the ambient environment and the plant tissue. These interstices are smaller than .01 micrometers so as to preclude the passage therethrough of even the smallest microorganisms, such as viruses. It also provides rigidity to facilitate the transfer and handling of the cultures.

For practicing the invention described herein, it is preferred that the outer membrane material have a permeability to CO_2 of from 200 to 1190 cc/100 sq. in./24 hours at 1 atm. and a permeability to O_2 of from 100 to 424 cc/100 sq. in./24 hours at 1 atm. Chevron HiD-9650, the preferred material for the outer membranes, in the preferred 1.25 mil thickness, has a permeability to CO_2 of approximately 450 cc/100 sq. in./24 hours at 1 atm. and a permeability to O_2 of approximately 190 cc/100 sq. in./24 hrs. at 1 atm.

Other polymeric materials may be used which have greater permeability than the preferred high density polyethylene; however, if the permeability is too great, water within the membrane vaporizes and passes through the outer membranes and out of the integument. Thus, the moisture vapor transmission rate (MVTR) is an important factor in the selection of materials for the outer membranes. It is preferred that the outer membranes have a MVTR of from 0.2 to 0.684 gm/100 sq. in./24 hrs. at 1 atm. The preferred material, Chevron HiD-9650, in the preferred thickness of 1.25 mils, has a MVTR of 0.3 gm/100 sq. in./24 hours at 1 atm. Outer membranes of other materials, thicknesses, and permeabilities can be used depending upon the length of time the culture is to be grown in that integument. For example, the longer the growing period required for the particular plant material, the lower the MVTR of the material should be so as to prevent the media from drying out to a degree that it will not be conducive to growth. It is also noted that because in many applications the inner containers house media, the material, thickness and permeabilities of the membranes which form the inner containers must also be adjusted to achieve a desirable MTVR and prevent drying out of the media they house.

The preferred high density polyethylene for the outer membranes is high-density polyethylene No. HiD-9650, manufactured by the Chevron Chemical Company of Orange, Texas. Hi-D 9650 resin is a dry, fluffy powder as it exits the reaction process. This powder is run through a compounding extruder where it is melted and mixed with appropriate additives. As the 9650 polymer exits the compounding extruder, it is formed into round circular pellets which are subsequently packaged and shipped to customers for the manufacture of film through an extrusion process. Chevron has been manufacturing and selling a high density polyethylene resin designated as Chevron Hi-D 9650 since approximately 1984. Its prior uses include the manufacture of notion and millinery bags and as barrier packaging.

As set forth in Chevron "Technical Data Sheet", CCO 9650-286 dated February 1986 and Chevron brochure "Chevron Polyethylene Resins for Extrusion", CCO 50001-885 dated August 1985, both incorporated herein by reference, the Hi-D 9650 resin includes the following nominal properties:

(a) Melt index of 0.3 grams per 10 minutes;

(b) Density of 0.950 grams per cubic centimeter;

(c) Natural color; and

(d) Vicat softening point of 257°F.

Hi-D 9650 polyethylene film is manufactured from the Hi-D 9650 resin using the blown film extrusion process which is standard in the industry. Hi-D 9650 blown film having a nominal film thickness of 1.0 mils includes the following nominal properties:

(a) Spencer Impact of 3.2 inch-pounds per mil;

(b) 26 inch dart impact strength, F-50 of 90 grams per mil;

(c) Elemndorf tear, notched, MD/TD of 16/400 grams per mil;

(d) Tensile strength at break MD/TD of 7400/6300 psi;

(e) Elongation MD/TD of 460/650%;

(f) 1% Secant Modules MD/TD of 103,000/120,000 psi; and

(g) Moisture vapor transmission rate of 0.35 grams per 100 square inches per 24 hours per mil.

Chevron Hi-D 9650 is a high density, linear polyethylene copolymer resin made by the Phillip's loop reactor slurry process as depicted in Figure 8. Like all polyethylenes, Chevron Hi-D 9650 is a polymer of ethylene gas. To create Chevron Hi-D 9650, the ethylene gas can be reacted as a mixture with a comonomer, such as hexene or butene; hydrogen as a polymerization terminator; and a catalyst initiator. The method of using the system of Figure

8 in this process is well known to those skilled in the art. The reaction results in a long chain, slightly branched molecule of ethylene and the comonomer. The density of Hi-D 9650 is achieved by the amount of comonomer that is reacted with the ethylene. The greater amount of the comonomer, the lower the density of the resulting polyethylene.

The recommended commercial processing conditions for converting Hi-D 9650 resin to a film with a nominal film thickness of 1.0 mil, includes a melt temperature of 430°F and a die gap of greater than 0.035 inches. A smaller die gap may be used.

The gas transmission rates of water vapor, oxygen and carbon dioxide for various thicknesses of the preferred high density polyethylene (Chevron HiD-9650); another high density polyethylene (Chevron-9506); and a low density polyethylene (Chevron-PE5754) are included in Figures 10-12. The results are also in tabular form below. As can be seen, the thinner the membrane, the greater its transmission rates for these gases. In addition, it can be seen that for the three membrane materials tested, the low density material (Chevron PE-5754) has the highest transmission rate for each of these gases. Although gas exchange is important to the growth of plants within the integuments of the

invention, the preferred membrane made from Chevron's HiD-9650, has a low moisture vapor transmission rate which is lower than the other materials tested as shown in the graphs in Figures 10-12. As noted above, if the moisture vapor transmission rates are too great, the media in the integument will dry out too quickly and the biological material therein will desiccate and die.

TABLE IV

MOISTURE VAPOR TRANSMISSION RATES
CARBON DIOXIDE TRANSMISSION RATES
OXYGEN TRANSMISSION RATES

SECTION I. MOISTURE VAPOR TRANSMISSION RATES
GRAMS/100 IN.SQ./24 HRS.

RESIN I.D.	Hi-D 9650		Hi-D 9506		PE 5754	
TARGET FILM GAUGE, MILS.		ACTUAL GAUGE		ACTUAL GAUGE		ACTUAL GAUGE
0.50	1.190	0.570	1.261	0.590	2.200	0.600
0.75	0.684	0.840	0.932	0.850	1.358	0.720
1.00	0.432	1.010	0.623	1.000	1.035	1.050
1.20	0.339	1.230	0.455	1.200	0.897	1.250
1.50	0.223	1.480	0.320	1.630	0.700	1.450

SECTION II. CARBON DIOXIDE TRANSMISSION RATES
CC/100 IN.SQ./24 HRS.

RESIN I.D.	Hi-D 9650		Hi-D 9506		PE 5754	
TARGET FILM GAUGE, MILS.		ACTUAL GAUGE		ACTUAL GAUGE		ACTUAL GAUGE
0.50	2093.5	0.525	2031.5	0.550	3087.5	0.500
0.75	1190.5	0.750	1396.5	0.800	2281.0	0.775
1.00	620.0	1.000	886.0	1.050	1587.5	1.050
1.20	450.5	1.225	622.0	1.225	1407.5	1.175
1.50	339.0	1.450	463.5	1.525	1246.5	1.500

SECTION III. OXYGEN TRANSMISSION RATES
CC/100 IN.SQ./24 HRS.

RESIN I.D.	Hi-D 9650		Hi-D 9506		PE 5754	
TARGET FILM GAUGE, MILS.		ACTUAL GAUGE		ACTUAL GAUGE		ACTUAL GAUGE
0.50	1143.5	0.535	863.1	0.645	1556.5	0.625
0.75	424.4	0.795	746.0	0.745	1089.5	0.760
1.00	238.0	0.960	464.0	1.000	707.5	0.965
1.20	215.0	1.200	283.0	1.200	584.5	1.240
1.50	140.0	1.530	215.0	1.450	458.5	1.540

Having described the integuments generally and their uses, specific applications therefor will now be addressed.

Inoculation of Legumes

Legumes are plants which bear their seeds in pods, as for example, beans and peas, and are used for human or animal nutrition. Legumes include seed, hay, silage, winter cover crops, cash crops, and pasture. Legumes are rich in high quality protein, and in phosphorus and calcium. They are also a good source of vitamins, especially vitamins A and D. The nitrogen and the protein content of the legume is related because nitrogen is an element needed in the biosynthesis of protein.

Legumes can be inoculated with legume bacteria in order to enable the plants to fix, or change into usable form, atmospheric nitrogen. Bacteria of the genus rhizobia, including Rhizobium japonicum, Bradyrhizobium, and Azorhizobium can establish a symbiotic relationship with the legume to allow

fixation of nitrogen. The plant supplies sugar to the bacteria for energy, and the bacteria uses the energy from the sugar to fix nitrogen.

Inoculation is usually accomplished by mixing legume seeds with an appropriate strain of rhizobia before the seeds are planted. Conventionally, this process is carried out in the open environment. Seeds and rhizobia are conventionally mixed with soil in bulk, or sprayed on the soil, or the seeds are pre-coated with inoculant in bulk. It is also possible, though usually less efficient, for endogenous strains in the soil to inoculate the legume.

After the inoculated legumes are planted and begin to grow, the legume bacteria invade the root hairs. Nodules are formed on the roots, and the bacteria occupy the nodules.

Nitrogen fixation increases the overall nitrogen content of soil. This can be useful if, after legumes have been grown, one wishes to grow other crops in the same soil.

Not all legume bacteria, however, are beneficial. Some do not aid in nitrogen fixation. The best bacteria for legume inoculation are those that compete with other bacteria for nodule sites on the roots of a growing plant. Such nitrogen-fixing

bacteria are more likely to prevail for nodule sites on the roots. Because the conventional mixing of seeds and rhizobia is carried out in the open environment, contamination of nodule sites by undesired bacteria can be a problem.

In order to reduce the amount of inoculant required, it is usually recommended that the seeds and the rhizobia cultures be mixed with water, syrup, or a sugar solution prior to inoculation. The water, or solution, causes the inoculants to adhere to the seeds better than when the inoculants are used without water. The seeds should be planted soon after inoculation.

The integument 10 of the present invention can be used in legume inoculation. A solution of inoculant and sugar or syrup, or a frozen concentrate of these items, is housed within one or more of the inner containers. Alternatively, inoculant is housed in a first inner container, and water, syrup or another type of binder is housed in another one of the inner containers. The seed is placed inside the outer integument 10. In yet another alternative, with the seed inside outer integument 10, water or any media which induces germination could be placed in a first inner container, and inoculant and a binder could be

placed in one or more of the other inner containers 1, 2, 3, 4.

The inner container(s) with the inoculant or inoculant/binder can be broken and opened when desired to allow their contents to come in contact with the seeds. This can be done before the seeds are planted. Alternatively, if water is housed in one of the inner containers with inoculant/binder in another inner container, the seeds can first be induced to germinate by breaking the inner container holding the water. Then, once germination takes place, the other container(s) can be broken to release the inoculant and/or binder.

Following introduction of the inoculant/binder, the seeds and the inoculant/binder are then mixed by vigorously rotating, shaking, and/or massaging the integument. One advantage of allowing the seeds to germinate before inoculation is that the inoculant comes into contact with the root hairs and the stems where the nodules actually form. This can allow more efficient use of a smaller amount of inoculant. In addition, less soil preparation may be needed with the inoculant positioned as such. It is noted that in the conventional inoculation systems, the seeds are not usually allowed to germinate before

inoculation, and therefore the advantages obtained with the system of the invention is not achieved.

It should also be noted that rather than mixing the seeds and inoculant/binder in the sealed integument, they can be mixed after the integument is opened. In either case, once mixing is completed, the integument is opened and the inoculated seeds are sown.

Because the integument 10 of the invention is contaminant impermeable, its contents, i.e., the seeds and the inoculants, remain sterile before the inoculation step. This allows the farmer to carry out on-site inoculation (and/or germination) without fear of contamination, as in the conventional system which is open to the environment. Further, the amount of wasted inoculant can be reduced because it can be pre-measured to suit the amount of seed in the integument. In addition, the inoculant tends to be more effective, in that more of it adheres to the seeds and performs the inoculation function in the sealed integument. In the conventional systems, where mixing is carried out in bulk, or the inoculant is sprayed on the soil, less of the inoculant will end up at the nodule site where it is needed. The system of the invention results in a savings of time

and money to the farmer, who uses less inoculant, and also in greater convenience.

Plant Tissue Culturing

Micropropagation of plants, for which tissue culturing is essential, involves four basic stages:

Stage 1 Initial Tissue Culturing

Stage 2 Tissue Culture Multiplication

Stage 3 Differentiation and Plant Formation

Stage 4 Greenhouse Culturing and Hardening

These stages, and the overall micropropagation process, are described in detail in my co-pending application Serial No. 021,408 filed March 4, 1987, and entitled "Integument and Method for Micropropagation and Tissue Culturing."

The culture progresses from stages 1 to 3 by changing the growth media. At stage 4, the plantlets are removed from the sterile environment and transferred to a greenhouse.

In micropropagation, a piece of undifferentiated tissue, or meristematic tissue, is removed from the parent plant or cultivar and cultured to produce identical offspring. The main problem in tissue culturing is contamination.

Precautions are taken to reduce contamination at the first three stages of the process. The tissue is washed following removal, and all media and equipment

is sterilized. In addition to covering the containers which contain the culture, the cultures are grown in a culture room with a filter which removes most airborne microorganisms.

As discussed in copending Application Serial No. 021,408, contamination can be further reduced by carrying out stages 1 to 3 in integuments which are gas-permeable and contaminant impermeable. In addition to advantages such as cost, lack of fragility, and space savings, use of integuments also results in a substantially enhanced growth rate for the cultures.

The integument/container system of the present invention, particularly integument 40 shown in Figure 5, can be advantageously employed in tissue culturing. A measured amount of the growth media needed for the cultures in each of the stages 1 to 3 can be pre-packaged in the inner containers, e.g., inner containers 62, 64, 66, in three different chambers, e.g., 42, 44, and 46. The cultures are placed in the chambers. The system is completely sterilized.

When one wishes to initiate stage 1 growth, one simply breaks the inner container, which contains a suitable medium for stage 1 growth, e.g., Murashige Minimal Organic Medium manufactured by Carolina

Biological Supply Co. This can be done by applying pressure from outside the integument in the area of the inner container. Because the amount of medium is pre-measured, the integument/container can be shipped with the culture and medium inside and culturing can be initiated at the time and place desired.

After stage 1 growth has been completed, the culture can be transferred to an another chamber under sterile conditions, e.g., under a laminar flow hood. The inner container in this chamber contains the medium needed for stage 2 growth, e.g., Murashige Shoot Multiplication Mediums A, B, and C, available from Carolina Biological Supply Co. The inner container is broken as before once one desires to initiate stage 2 growth. The advantage over the usual system wherein media is added to the integument is that the media need not be handled at the time it is added to the tissue. The risk of contamination is thereby reduced. Additionally, as before, the amount of media is pre-measured to reduce errors and aid in conservation of media.

Following stage 2 growth, the culture is transferred as before to another chamber which contains a medium suitable for stage 3 growth, e.g., Murashige Pre-Transplant Mediums, available from

Carolina Biological Supply Co. The steps outlined above are followed.

It can be seen that this system provides several advantages, including conservation of media, reducing the possibility of contamination, and the ability to initiate culture growth at a predetermined place and time. An alternative system includes three (or more) different media needed for one or more of stages 1 to 3 growth in three different inner containers, all housed within one integument, for example, integument 12 of Fig. 1. The inner containers are broken at the appropriate times to initiate the various stages of growth. This latter system works especially well if the media for stages 1 and 2 growth are in the inner containers. The culture would be transferred to another integument for stage 3 growth. The advantage of such a system is that stage 1 growth does not require any hormones, and the stage 1 medium does not contain any. Thus, if the inner containers house the stage 1 and 2 media, when they are later broken to contact the culture there will not be any undesired mixing of hormones.

An alternative system has a tissue callous, protoplast, or a small piece of tissue in an inner container of integument 10. The inner container also houses an initiation media. A second media is

outside the inner container and within the outer membrane 12. Once the tissue sample in the inner container has grown to sufficient size, the inner container is broken and the sample comes in contact with the second media, which induces the next phase of growth. After completion of the second growth phase, the sample can be removed from the integument 12 and placed in stage 3 media, as described above. In the alternative, another inner container in integument 12 contains the stage 3 growth media, can be broken to initiate stage 3 growth.

A variation on the basic integument system described above has an outer membrane, an inner container, and an innermost container within the inner container. The inner container can contain, for example, the tissue culture, with the innermost container housing a stage 1 growth media for the tissue culture. The outer membrane houses a stage 2 growth media. The innermost container is broken to initiate culture growth, and, once stage 1 growth is completed, the inner container is broken to bring the culture into contact with the stage 2 growth media. It can be seen that variations on this system could have additional containers with other growth media housed within the innermost container. These could

be broken to accomplish various stages of culture growth.

An integument 10 of the invention can also be used in tissue culturing a plant or legume which is to be inoculated. The media for one or more of stages 1 to 3 growth is housed in a set of inner containers, and another container, or containers, contains the inoculant and binder. The culture is grown in the integument casing by adding the various media at appropriate times. After the culture has developed, preferably to stage 3 where stem and root formation takes place, the inoculant and binder are added. The culture and inoculant/binder are then shaken as before to ensure thorough inoculation.

This system allows the plantlet or culture to be inoculated at a stage of development such that only a minimum amount of inoculant is needed, because it is applied directly to developing stems and roots. In addition to use in legume inoculation, this system could be used in inoculation of other plants by bacteria or microorganisms which affect the plants. For example, certain bacteria can enhance the growth rate of certain plants. In such a system, plants are inoculated by these bacteria at an appropriate stage of plant development. The advantages are that inoculation is carried out in a closed system, and

that it can be done at a development stage where the bacteria is most efficiently used to accomplish the purpose for which it is added. Seed Propagation

The integument/container system of the invention may also be used advantageously where a plant is grown from seed, which process is known as seed propagation. See U. S. Application Serial No. 021,408. Referring to Figures 6 and 7, growth medium is place in inner container 110 of integument 100, which is of sufficient dimensions to house a fully grown plant. The seeds are placed outside the inner container 110 but within the lower chamber 104 of integument 100.

The growth medium can be, for example, Murashige Minimal Organic with 30 grams of sucrose and 8 grams of agar dissolved in a total volume of 1000 ml of distilled water by the methods described in U. S. Application Serial No. 021,408, with the pH adjusted to 5.5. This medium is well suited to growing lettuce, and especially Black-seeded Simpson lettuce. Once one desires to initiate growth, the inner container is broken and opened to mix the medium with the seeds and initiate growth. The medium flows down into the lower chamber 104 where the seeds are located.

As discussed in U. S. Application Serial No. 021,408, seed propagation in an integument leads to an increased rate of growth and reduces loss through contamination, in the case where one is growing a complete plant (or lettuce head) from seed. These same advantages follow from using the integument/container system of the present invention, with the added advantage that growth can be initiated at a predetermined time and place, and that the medium need not be handled at the time one initiates growth. This conserves the medium and reduces contamination.

An alternative to growing a complete plant in an integument is to merely germinate the seeds in the integument, and then plant them in an open environment shortly thereafter. In such a case, an inner container could contain water and the nutrients essential for plant growth, i.e., Molybdenum, Copper, Zinc, Manganese, Iron, Boron, Chlorine, Sulfur, Phosphorous, Magnesium, Calcium, Potassium, Nitrogen, Oxygen, Carbon and Hydrogen. This process could advantageously be performed, for example, in integument 40 of Figure 5.

Soil, peat moss or another type of non-sterile substrate can be housed inside the chambers, and desiccated seeds are placed on this substrate. When

one wishes to initiate growth, the inner containers are opened, and the water/nutrients contact the seeds and cause germination. Contamination is not a concern in this system, because the plant is to be exposed to the environment almost immediately after germination.

The advantage of the integument in the germination system is that germination can be initiated at any time, water loss is controlled by the outer membrane and this results in a controlled germination and less of a risk of post-germination desiccation. The outer membrane also provides the gas exchange needed for growth. An alternate integument in the germination system can include a contaminant-permeable outer membrane made, for example, of low density polyethylene. The advantage would be higher gas exchange to enhance plant growth and, even though water loss would increase somewhat, this could be compensated by including more water in the inner containers.

Inoculation of plants grown from seeds in an integument may also be carried out in an integument. Legumes, which are grown from seeds in an integument, may be inoculated or any type of plant may be inoculated by any bacteria. In these systems, for which integument 10 of Figures 1-4 is well-suited,

the inoculant or inoculant/binder is housed in one or more inner containers, and the outer membrane houses the seed. Another inner container, or containers, houses the media or water. Once the media/water and seed are mixed, as described above, and the plant is at an appropriate stage of development, the inner container(s) is broken to inoculate the seed or plant. The advantages are the same as those that follow with the inoculation of a plant grown from tissue culture.

Cellulose Production

Amongst others, the bacteria known as Acetobacter can produce cellulose. Acetobacter grows best in a liquid nutrient broth. A fixative, for example, glutaraldehyde or another of the formalin/aldehydes, can be added to stop the growth of Acetobacter. The fixative also fixes all the cell components so that they can be further treated, stained and then studied. In this manner one can determine which cell processes were in progress at the time of fixation. This information is useful in the study of cellulose production.

It is desirable to produce cellulose in a near zero or low gravity environment, such as in an orbiting spacecraft. Cellulose produced under these conditions has a different crystalline structure and

as a result may be stronger than that produced in stronger gravitational fields. Such cellulose can be produced advantageously with the integument/containers of the invention.

In such a system, the Acetobacter is placed inside the outer membrane of, for example, integument 10 or integument 40, and an inner container houses the fixative. Alternatively, another inner container, or containers, can contain a growth medium for the Acetobacter.

After the growth medium has acted on the Acetobacter and sufficient cellulose has been produced, the inner container with the fixative is broken or opened to halt further production.

This system can be used in several applications, and particularly in experiments to measure the stage of Acetobacter development at which cellulose growth is optimal. In such an experiment, the Acetobacter is grown through several stages and growth is arrested at each stage by breaking the inner container to determine the stage where cellulose production was highest.

Another type of experiment for which the system can be used is one wherein changes in crystal structure are being measured. Acetobacter are first grown in an integument in a normal gravitational

field and then transferred to a near zero environment. Cellulose production is arrested at various stages after introduction to the low gravity environment and the crystals produced are examined. In this way it can be seen at which stage the change in crystal structure takes place.

It should be understood that the terms and expressions used herein are exemplary only and not limiting, and that the scope of protection is limited only by the claims which follow and include all equivalents of the subject matter of those claims.

What Is Claimed Is:

1. An apparatus for mixing a selected substance with a biological product, comprising:

a cellule formed of a gas permeable, liquid impermeable membrane adapted for enclosing the biological product;

at least one compartment for enclosing the substance which affects the biological product, said compartment disposed within said cellule and adapted to be opened from outside said cellule without breaching the integrity of said cellule.

2. The apparatus of claim 1 wherein said cellule has a non-artificial internal and external pressure, the gas mixture and pressure within said cellule and the gas mixture and pressure of the atmospheric ambient environment equalizing by gas exchange through said membrane, said membrane being completely sealed around the biological product and the substance for preventing exposure of the biological product and the substance to biological contaminants in the atmospheric ambient environment.

3. The apparatus of claim 1 wherein said membrane is made of high density polyethylene.

4. The apparatus of claim 1 wherein the membrane has a moisture vapor transmission rate equal to that of a high density polyethylene film with a thickness in the range of 1 to 2 mils.

5. The apparatus of claim 1 wherein the membrane is a single layer of material.

6. The apparatus of claim 4 wherein the moisture vapor transmission rate of the membrane is less than 0.68 grams per 100 square inches per 24 hours at 1 atmosphere.

7. The apparatus of claim 1 wherein said membrane can withstand a temperature of 250°F at a pressure of 15 psi without deformation.

8. The apparatus of claim 6 wherein the moisture vapor transmission rate is between 0.2 and 0.68 grams per 100 square inches per 24 hours at 1 atmosphere.

9. The apparatus of claim 1 wherein said membrane transmits light rays therethrough having a wave length of between 400 and 750 nanometers.

10. The apparatus of claim 1 wherein said cellule is formed of a membrane made of high density polyethylene.

11. The apparatus of claim 1 wherein said membrane is Chevron high density polyethylene 9650.

12. The apparatus of claim 1 wherein said membrane has a carbon dioxide transmission rate of between 200 and 1190 cubic centimeters per 100 square inches per 24 hours at 1 atmosphere.

13. The apparatus of claim 1 wherein said membrane has an oxygen transmission rate of between 100 and 424 cubic centimeters per 100 square inches per 24 hours at 1 atmosphere.

14. An apparatus for affecting a biological product, comprising:

a gas permeable and liquid impermeable membrane forming a cellule for enclosing the biological product and a substance which affects said biological product, said membrane forming a first chamber which is completely sealed around the biological product for preventing exposure of the biological product to biological contaminants in the ambient environment, said membrane enclosing a second material and said second material forming at least one sealed compartment within said membrane, said membrane being made of high density polyethylene which will permit oxygen and carbon dioxide to diffuse therethrough, said sealed compartment adapted to be opened without opening the first chamber.

15. The system of claim 14 wherein the second material is also made of high density polyethylene.

16. The system of claim 14 wherein the second material is made of low density polyethylene.

17. A system for affecting a biological product, comprising:

a biological product;

a substance for affecting said product;

a gas permeable, liquid impermeable membrane made of lightly cross-linked layers of polymers and having a moisture vapor transmission rate equal to that of a high density polyethylene film with a thickness in the range from 1 to 1.5 mils, said membrane having interstices smaller than the size of a virus but large enough for gas exchange, said membrane forming a cellule for enclosing said biological product and surrounding and enclosing at least one inner integument which is adapted to enclose said substance, said cellule being completely sealed around the biological product for preventing exposure of the biological product to biological contaminants in the ambient environment, said inner integument adapted to be opened without opening said cellule.

18. The system of claim 17 wherein said inner integument is made of high or low density polyethylene.

19. The system of claim 17 wherein the inner integument is adapted to house a growth media for the biological product.

20. The system of claim 19 wherein there are two inner integuments, one being adapted to house an inoculant for the biological product and the other being adapted to house a binder to aid in attachment of the inoculant to the biological product.

21. A system for affecting a biological product, comprising:

a gas permeable and liquid impermeable membrane forming a cellule for enclosing the biological product, the gas mixture and pressure within the cellule being substantially the same as the gas mixture and pressure of the ambient environment upon enclosure, the cellule also enclosing at least one inner integument which is completely sealed from the cellule, said inner integument adapted to be opened without opening the cellule;

said membrane being completely sealed around the biological product for preventing exposure of the biological product to contaminants in the

ambient environment, said membrane having interstices smaller than a virus but large enough for gas exchange.

22. The system of claim 21 wherein the inner integument is made of a material which is liquid impermeable.

23. The system of claim 21 wherein the membrane is made of high density polyethylene.

24. The system of claim 21 wherein the inner integument is impermeable or semipermeable to water vapor.

25. The system of claim 21 wherein the membrane has a permeability to water vapor of 0.32 grams per 100 square inches per 24 hours.

26. A method of affecting a biological product at the same time as it is isolated from the outside environment, comprising:

enclosing the biological product in a gas permeable and liquid impermeable membrane, said membrane forming a first chamber which is completely sealed around the biological product for preventing exposure of the biological product to biological contaminants in the ambient environment, said membrane enclosing a second material and said second material forming at least one sealed compartment within said

membrane, said membrane being made of high density polyethylene which will permit oxygen and carbon dioxide to diffuse therethrough, said sealed compartment adapted to be opened without breaching the membrane;

enclosing a substance which affects said biological product in said second material.

27. The method of claim 26 wherein the biological product is a plant tissue culture and the substance is a tissue culture growth medium.

28. The method of claim 26 wherein the biological product is legume seed and the substance is a legume inoculant culture.

29. The method of claim 26 wherein the biological product is a plant seed and the substance is water or a nutrient media for the plant seed.

30. The method of claim 26 wherein the biological product is Acetobacter and the substance is a fixative for Acetobacter.

31. A method of affecting a biological product at the same time as it is isolated from the outside environment, comprising:

enclosing a biological product in a gas permeable and liquid impermeable membrane, said membrane forming a first chamber which is completely sealed around the biological product

for preventing exposure of the biological product to biological contaminants in the ambient environment, said membrane enclosing a second material and said second material forming at least one sealed compartment within said membrane, said membrane being made of high density polyethylene which will permit oxygen and carbon dioxide to diffuse therethrough, said sealed compartment adapted to be opened without opening said first chamber;

enclosing a substance which affects said biological product in said sealed compartment.

32. A method of inoculating legume seeds or a legume tissue culture, comprising:

placing the legume seed or legume tissue culture within the cellule of the apparatus of any of claims 1 to 13;

placing the inoculant or an inoculant and a binder in the compartment of the apparatus of any of claims 1 to 13;

breaking the compartment open from outside the integument without breaking the cellule.

33. A method of germinating a plant seed, comprising:

placing the seed within the cellule of the apparatus of any of claims 1 to 13;

placing water, or a solution of nutrients, in the compartment of the apparatus of any of claims 1 to 13;

breaking the compartment open from outside the integument and without breaking said cellule.

34. A method of producing cellulose, comprising:

placing an Acetobacter culture within the cellule of the apparatus of any of claims 1 to 13;

placing a fixative in the compartment of the apparatus of any of claims 1 to 13;

breaking the compartment open from outside the integument and without breaking said cellule.

35. A method of tissue culturing, comprising:

placing a tissue sample within the cellule of the apparatus of any of claims 1 to 13;

placing a growth media for the tissue sample in the compartment of the apparatus of any of claims 1 to 13;

breaking the compartment open from outside the integument without breaking said cellule.

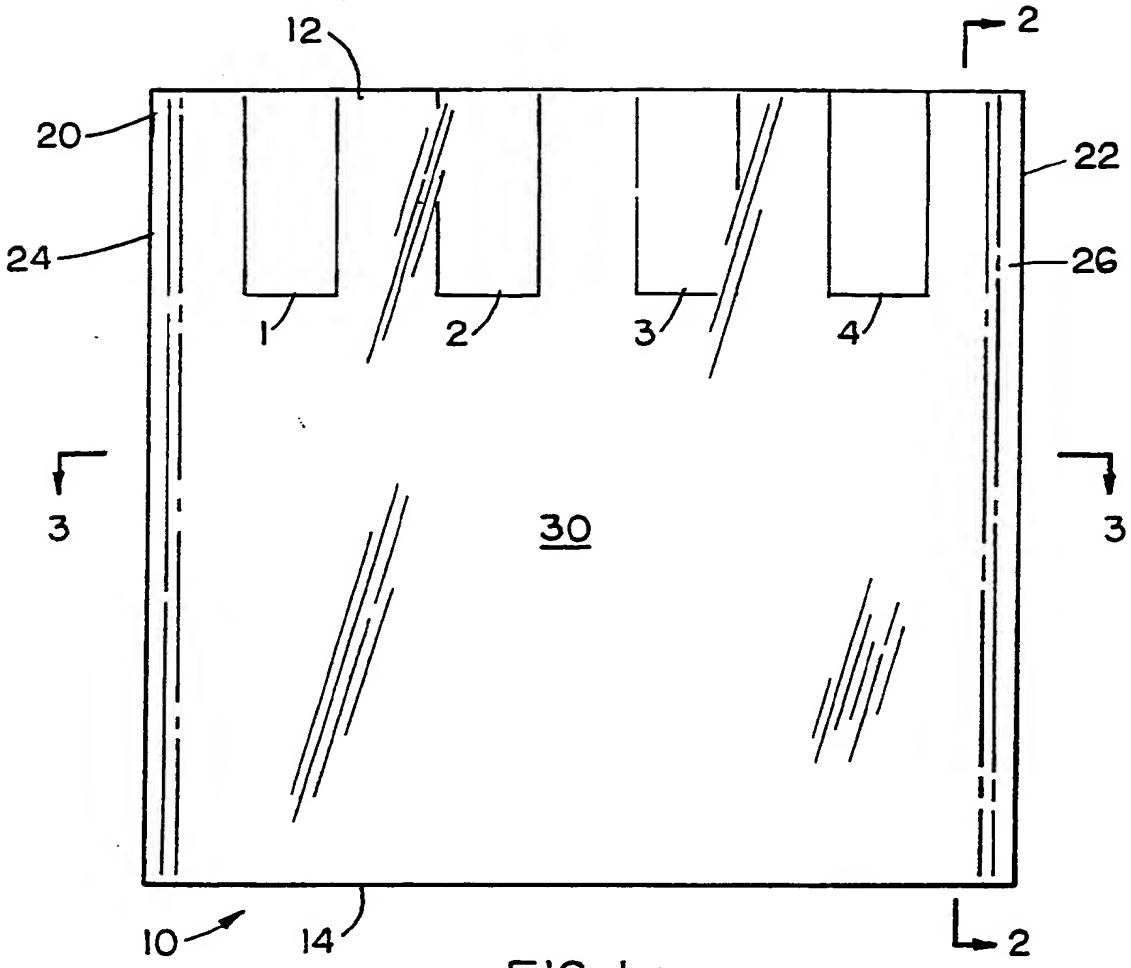

FIG. 1V

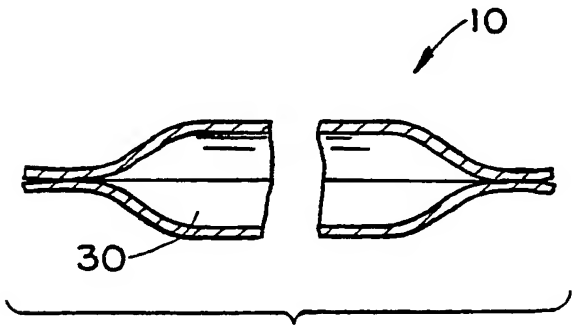

FIG. 3

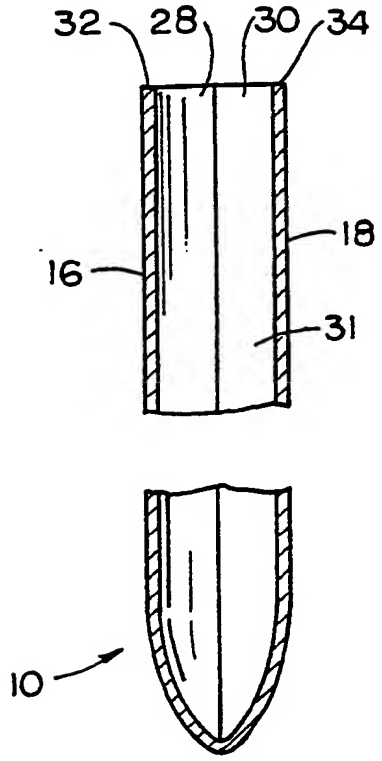

FIG. 2

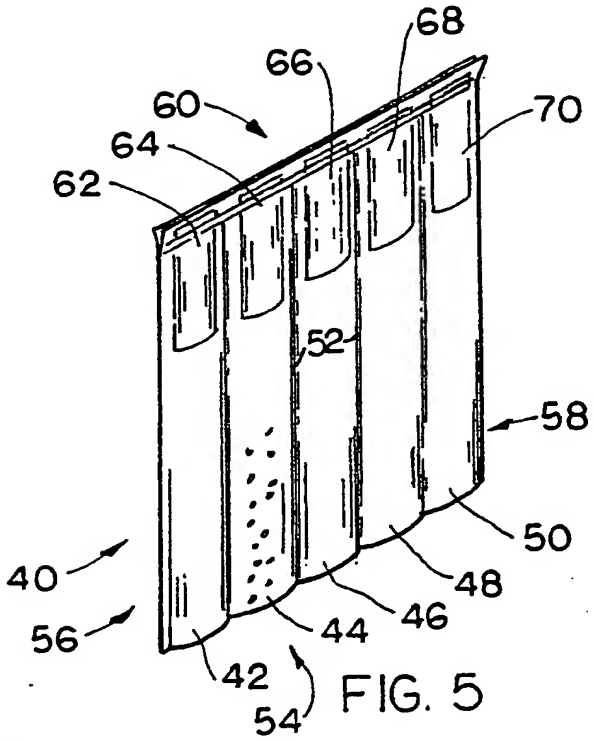

FIG. 5

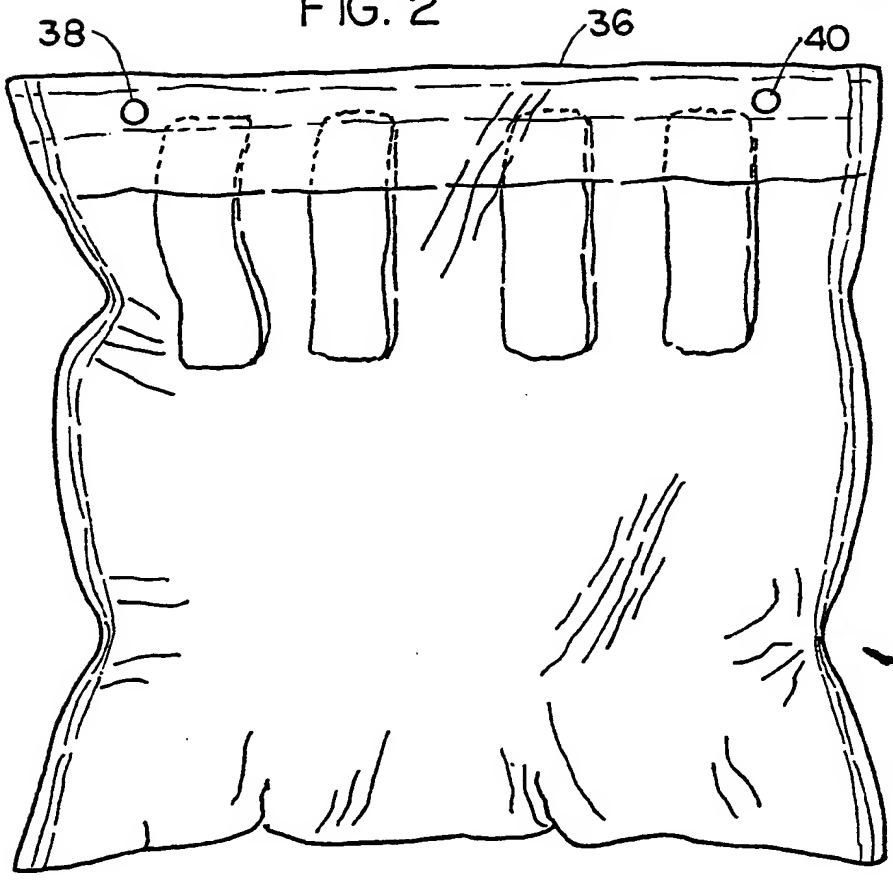

FIG. 4

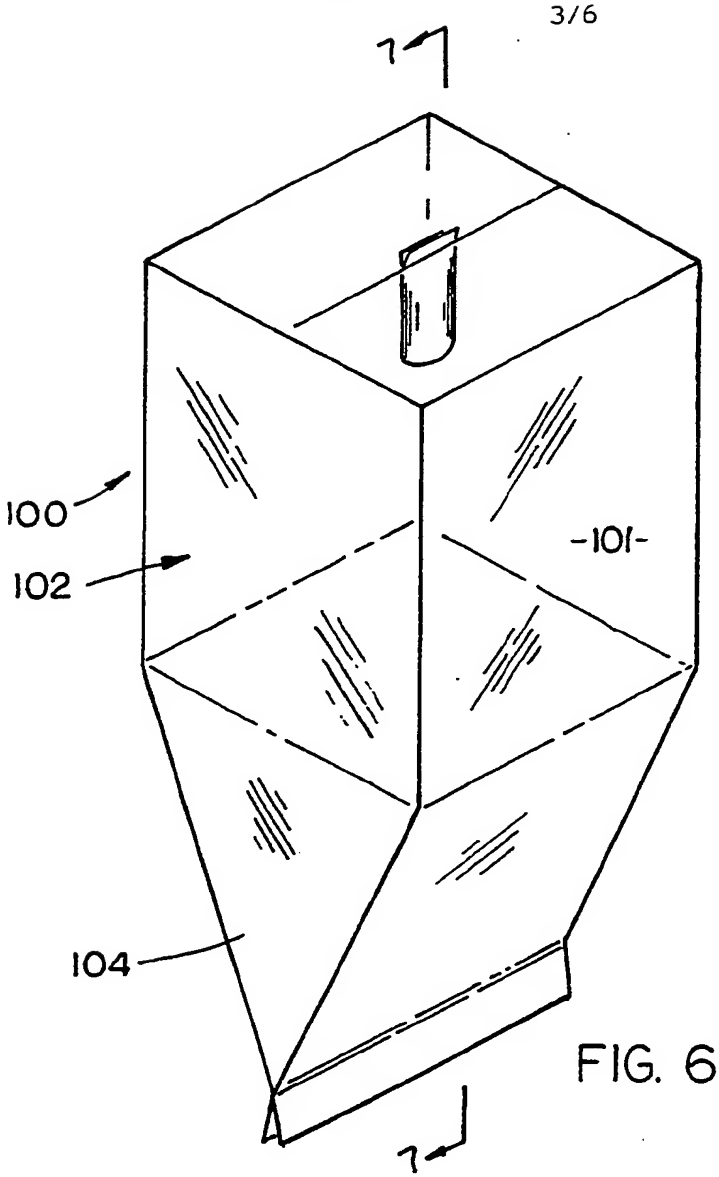

FIG. 6

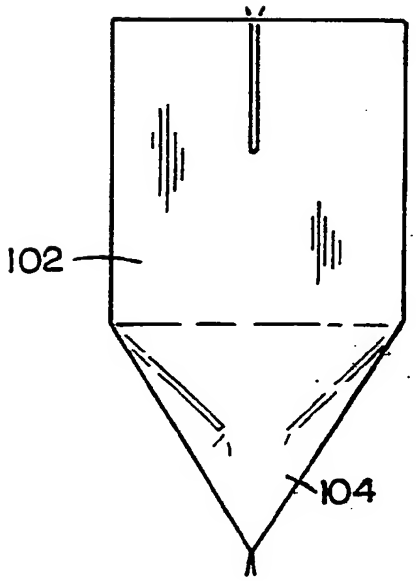

FIG. 7

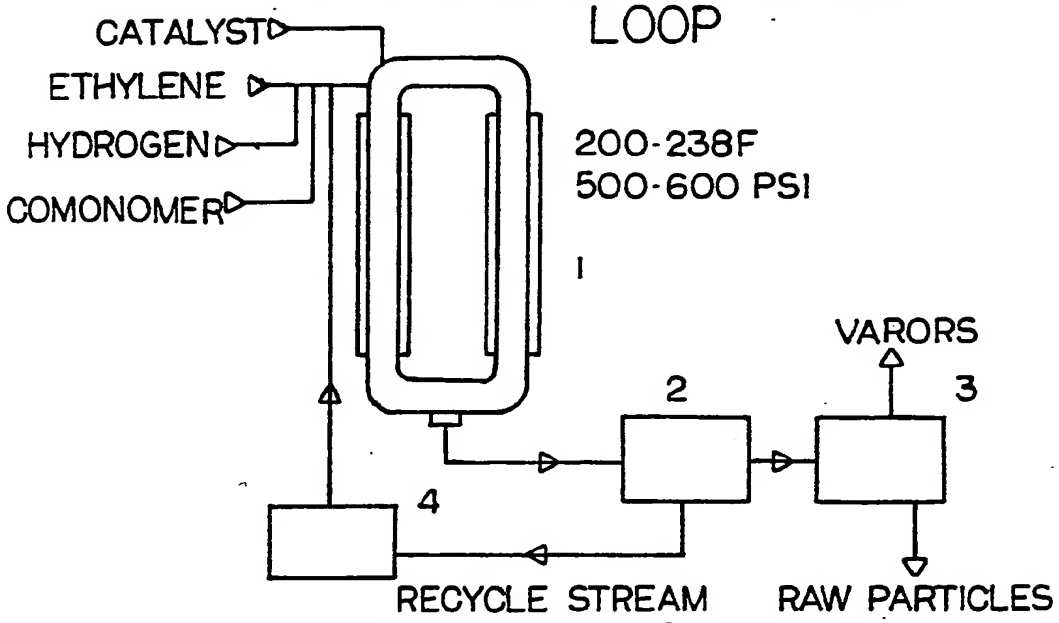

FIG. 8

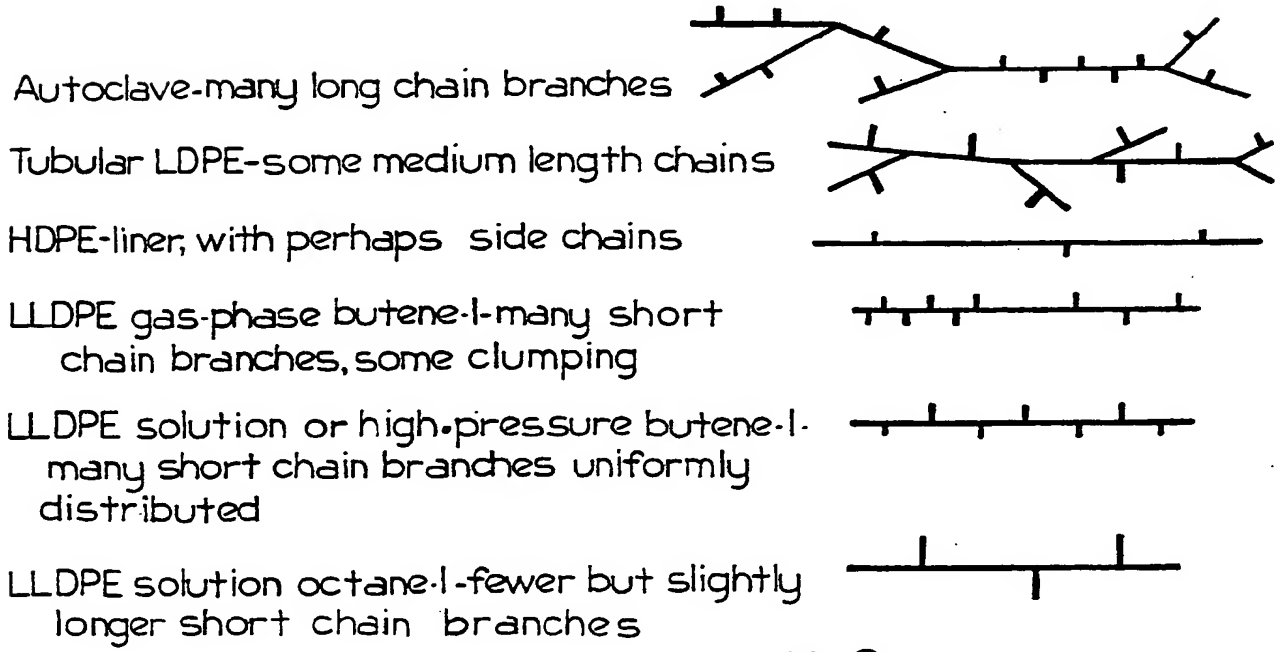

FIG. 9

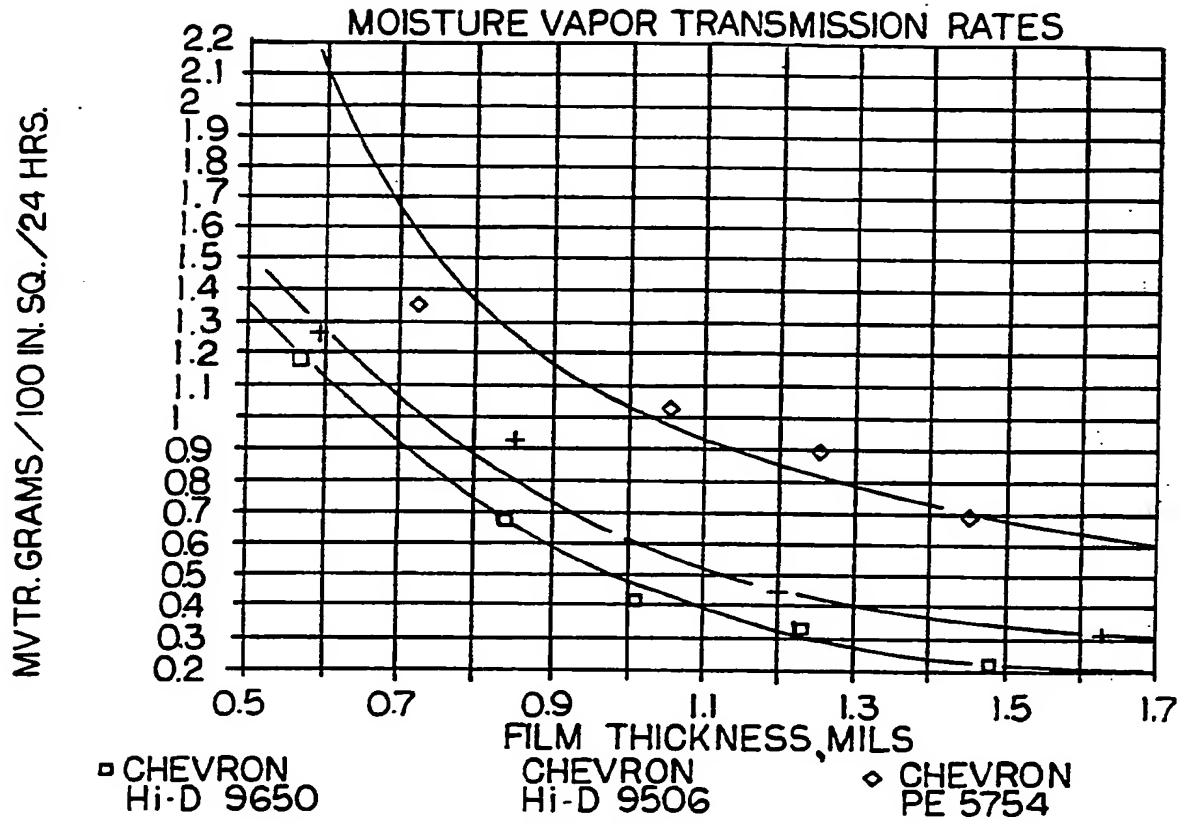

FIG. 10

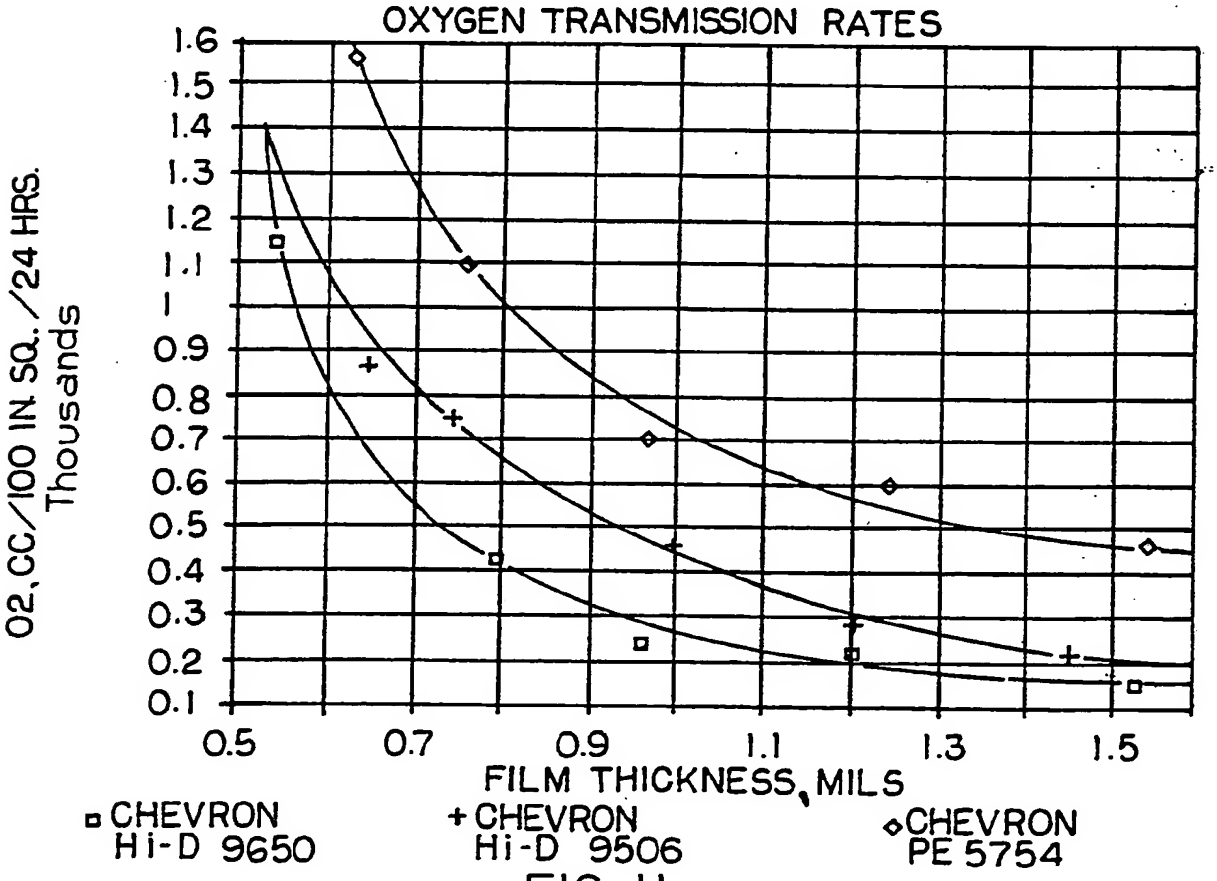

FIG. 11

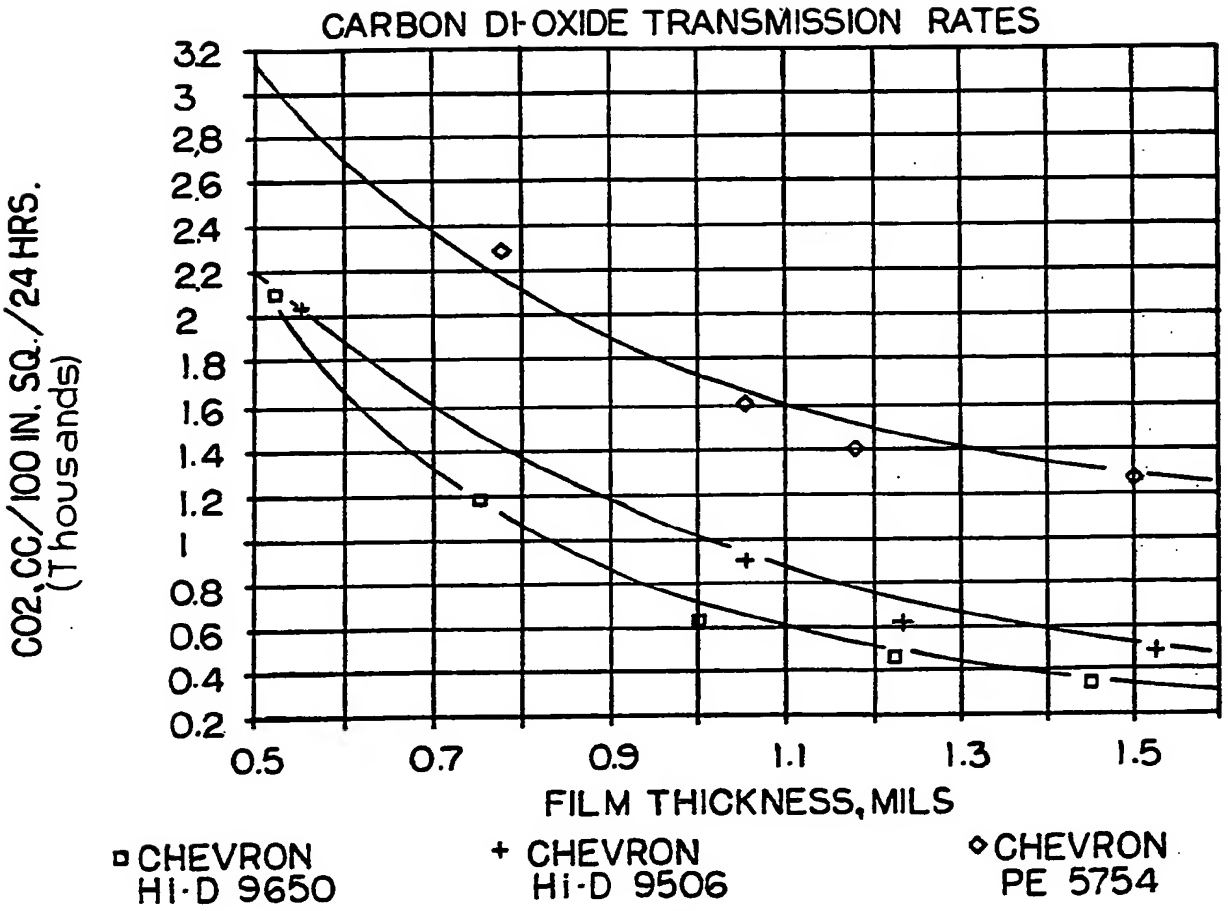

FIG. 12

INTERNATIONAL SEARCH REPORT

International Application No [redacted]/US90/02859

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): A01H 1/00; A01G 9/02; C05F 11/08; C12N 5/00; C12P 19/04; C12N 1/00
U.S.Cl.: 47/66; 47/84; 47/87; 71/7; 435/240.4; 435/101; 435/823, 47/58

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.Cl.	47/58,66,84,87 71/7 435/240.4,101,823

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [6]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	US, A, 3,320,697 (LARSEN) 23 May 1967, see column 2, lines 8-37.	1-35
Y	US, A, 4,539,793 (MALEK) 10 September 1985, see column 1, lines 23-41.	1-35
Y	US, A, 3,314,194 (HALLECK) 18 April 1967, see column 4, lines 12-20.	1-35
Y	US, A, 3,323,640 (KUGLER) 06 June 1967, see column 2, lines 14-40.	1-35
Y	US, A, 4,400,910 (KOUDSTAAL et al.) 30 August 1983, see column 1, lines 49-60, column 2, lines 7-11.	1-35
Y	US, A, 3,961,444 (SKAIFE) 08 June 1976, see column 2, lines 45-58.	1-29, 31-33
Y	US, A, 4,711,656 (KANESHIRO) 08 December 1987, see column 5, lines 20-36.	28, 32
Y	US, A, 4,463,522 (LINDEMANN) 07 August 1984, see column 2, lines 20-43.	27, 35

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
16 August 1990	09 OCT 1990

International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	for David T. Fox NGUYEN NGOC-HO INTERNATIONAL DIVISION

Form PCT/ISA/210 (second sheet) (May 1986)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	US, A, 3,184,395 (BREWER) 18 May 1965, see column 1, lines 37-50.	30, 34
Y	US, A, 4,745,058 (TOWNSLEY) 17 May 1988, see column 1, lines 50-68, column 2, lines 1-10.	30, 34
Y	US, A, 4,788,146 (RING et al.) 29 November 1988, see column 3, lines 40-50.	30, 34
Y	"Chevon Chemical Company Technical Data Sheet," published February 1986, by Chevron Chemical Company (Houston, Texas, USA), see entire document.	1-35

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . . because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers . . . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
Y	Biotechnology Letters, Volume 7, Number 7, issued July 1985, (Kew, England), Kybal et al., "A device for cultivation of plant and animal cells," pages 467-470, see especially page 468.	27, 35
Y	US, A, 4,769,945 (MOTOYAMA et al.) 13 September 1988, see column 2, lines 26-48.	1-29, 31-33, 35

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